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Novel autoantibodies in Sjögren's syndrome: A comprehensive review

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ABSTRACT

Sjögren's syndrome is a systemic autoimmune disease characterized by immune- mediated injury of exocrine glands, as well as a diverse array of extraglandular manifestations. B cell over-activation is a key feature of the disease, attested by the wide spectrum of autoantibodies detected in these patients. Up to date, anti- Ro/SSA and anti-La/SSB antibodies are traditional biomarkers for disease classification and diagnosis. On the other hand, the detection of novel autoantibodies in SS has increased in the last years, opening a window of opportunity to denote particular stages of the disease, to establish clinical phenotypes, and to predict long-term complications such as lymphoma. For instance, anti-SP-1, anti-CA6 and anti-PSP antibodies occur in an earlier stage than anti-Ro/La antibodies, and may identify a subset of primary Sjögren's syndrome patients with mild or incomplete disease, whereas anti-cofilin-1, anti- alpha-enolase and anti-RGI2 antibodies are potential biomarkers of MALT lymphoma. Antibody detection is also important to elucidate new aspects of SS pathophysiology, and in the future to permit a phenotype-specific patient approach. Herein we review the literature regarding new autoantibodies in SS and attempt to dissect their usefulness as diagnostic tools, pathogenic role, identification of clinical phenotypes and as predictors of an overlap syndrome.

1. Introduction

Sjögren's syndrome (SS) is a systemic autoimmune disease characterized by immune-mediated injury of exocrine glands, mainly salivary and lacrimal glands, and a diverse array of extraglandular manifestations [1]. T cells comprise a large fraction of the inflammatory infiltrate of affected salivary glands. However, B cell over-activation is also a key feature of the disease, attested by the wide spectrum of autoantibodies detected in sera of these patients (e.g. antinuclear antibodies, anti-Ro/SSA, anti-La/SSB, rheumatoid factor) as well as the participation of the B- cell activator factor (BAAF) complex [2]. Moreover, the presence of ectopic germinal centers, mainly in salivary glands [3] but also in other affected organs [4], also highlights that B-cell activation is characteristic of the disease. Not surprisingly, patients with germinal centers have higher titers of autoantibodies (e.g. rheumatoid factor, anti-Ro/SSA) and a higher risk for development of lymphoma [3]. Furthermore, the presence of antibodies may precede the disease onset. In this sense, Theander et al found at least one autoantibody specificity (ANA, RF, anti-Ro 60/SSA, anti-Ro 52/SSA and La/SSB) in 81% of 117 primary SS patients up to 20 years before diagnosis, being the highest positive predictive values for anti-Ro60/SSA and anti-Ro52/SSA antibodies [5].

In this setting, some antibodies (e.g. anti-Ro/SSA and anti-La/SSB) have been useful for SS classification and diagnosis [6], others have been associated with a particular SS disease manifestation, such as anti-carbonic anhydrase 2 antibodies with renal tubular acidosis, while others are predictors of an overlapping autoimmune condition (e.g. anti-centromere, anti-mitochondrial antibodies).

Moreover, a pathogenic role is likely also for some of them (e.g. anti-Ro/SSA, anti- carbonic anhydrase, anti-muscarinic receptor antibodies) [7,8].

Previous reviews in SS have focused on anti-α-fodrin, anti-carbonic anhydrase-II, anti-muscarinic receptor antibodies, but mainly in anti-Ro/SSA, anti-La/SSB [6,8–11]. Briefly, anti-Ro/SSA and anti-La/SSB antibodies are traditional biomarkers of SS associated with glandular dysfunction, a higher prevalence of extra-glandular manifestations, hypergammaglobulinemia and other markers of B cell activation [6]. These antibodies have also been linked to cutaneous, neonatal and childhood- onset lupus [12,13], as well as congenital heart block (CHB), a disorder presumably caused by placental passage of maternal autoantibodies to Ro/SSA and La/SSB ribonucleoproteins which induce inflammation and subsequent fibrosis of the fetal atrioventricular node. Indeed, a possible link between vitamin D deficiency and anti-Ro/SSA and anti-La/SSB antibodies, as well as a higher seasonal frequency of CHB has been suggested [14].

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Table 1
Prevalence and association with clinical phenotypes of novel autoantibodies in Sjögren's syndrome.

Autoantibody	Study size	Prevalence (%)	Clinical associations	Reference
Anti-SP-1, anti-CA6 and anti-PSP	*n* = 89	40–67	Early disease, negative anti-Ro/SSA and anti-La/SSB serology.	18
	n = 134			19
	n = 123			20
Anti-IFI16	*n* = 133	29	Abnormal Schirmer's test, hyperglobulinemia, ANA+, germinal centers and higher FS	22
Anti-MDM2	*n* = 100	21	Longer disease duration, thrombocytopenia, anti-La/SSB +, hyperglobulinemia, higher ESSDAI	26
Anti-NA-14	*n* = 72	11.1	ANA negative, shorter disease duration, secondary SS	29
Anti-stathmin-4	n = 72	15.2	Polyneuropathy, skin vasculitis	31
Anti-PUF60	*n* = 84	29.8	ANA+, RF+, anti-Ro52, anti-Ro60 and anti-La/SSB+, anti-thyroid antibodies,hypergammaglobulinemia	33
Anti-NR2	*n* = 66	20	Memory dysfunction, depression	36
Anti-TRIM38	*n* = 235	10.2	Diminished lacrimal gland function, higher FS, anti-Ro52, anti-Ro60, anti-La/SSB, RF+, hypergammaglobulinemia	38
ASCA	*n* = 104	5	Triple positivity for anti-Ro52/SSA, anti-Ro60/SSA and anti-La/SSB, hypocomplementemia, cutaneous and lung involvement	40
Anti-calponin-3	*n* = 209	11	Neuropathy	41
Anti-gAChR	*n* = 39	23	Autonomic dysfunction	43
Anti-AQP1	*n* = 112	27.7	None	52
Anti-AQP5		76.8	Lower resting salivary flow	
Anti-AQP4	*n* = 109	10	NMOSD	50
Anti-aquaporins (AQP1, AQP3, AQP8, AQP9)	*n* = 34	38.2	Severe xerophthalmia	54
Anti P-selectin	*n* = 70	22.8	Thrombocytopenia	55
Anti-carbamylated proteins	n = 84	27	Correlation with total IgG, IgM, RF, β2-microglobulins, FS and germinal center-like structures.	56
Anti-moesin	*n* = 50	42	None	57
Anti-cofilin-1, anti-alphaenolase, anti-RG12	n = 70	–	MALT lymphoma	59
Anticitrullinated α-enolase	*n* = 60	15	Arthritis, higher pH urine levels	60
Anti-citrullinated α-enolase isotype IgA	n = 50	n = 50	Anti-Ro/SSA antibodies	61

ANA: antinuclear antibodies; AQ: aquaporin; ASCA: anti-*Saccharomyces cerevisiae* antibodies; CA6: carbonic anhydrase 6; ESSDAI: EULAR Sjögren's syndrome disease activity index; FS: focus score; gAChr: ganglionic acetylcholine receptor; IFI16: Interferon-inducible protein-16; MDM2: mouse double minute 2; NA-14: nuclear autoantigen 14 KDa; NMOSD: neuromyelitis optica spectrum disorder; NR2: *N*-methyl-D-aspartate receptor 2; PSP: parotid secretory protein; PUF60: poly(U)-binding splicing factor 60 KDa; RGI2: Rho GDP-dissociation inhibitor 2; RF: rheumatoid factor; SP-1: salivary protein 1; TRIM38: tripartite motif containing protein 38.

Recent research concerning the presence of novel antibodies is emerging in SS. Herein we review these novel antibodies (Table 1) and attempt to dissect their usefulness in four scenarios: as diagnostic tools, pathogenic role, identification of a clinical phenotype and as predictors of an overlap syndrome (Table 2).

2. Novel autoantibodies

2.1. Anti-salivary protein 1, anti-carbonic anhydrase 6 and anti-parotid secretory protein antibodies

As previously mentioned, autoantibodies may precede the onset of the disease by years. In this setting, three novel autoantibodies have been associated with preclinical and early SS: anti-salivary protein 1 (anti-SP-1), anti-carbonic anhydrase 6 (anti-CA6) and anti-parotid secretory protein (anti-PSP antibodies).

In the IL14α transgenic SS mouse model, Shen and cols observed overexpression of mRNA of several salivary gland proteins including SP-1, CA6 and PSP when compared to control C57BL/6 mice. Moreover, the autoantibodies against these proteins were present at the age of 6 months, stage when decreased salivary flow and autoantibodies deposition in salivary glands are already present, but before the occurrence of lymphocytic infiltration of salivary glands and anti-Ro/SSA and anti-La/SSB antibodies [15].

Table 2
Utility of novel autoantibodies in Sjögren's syndrome.

Autoantibodies	Diagnostic tool	Pathogenic role	Phenotype identification	Overlap syndrome
Anti-SP-1, anti-CA6 and anti-PSP	Yes	Uncertain	Yes	No
Anti-IFI16	No	Uncertain	Yes	No
Anti-MDM2	No	Uncertain	Yes	No
Anti-NA-14	Possible	Uncertain	No	No
Anti-stathmin-4	No	Possible	Yes	No
Anti-PUF60	No	Uncertain	Yes	No
Anti-NR2	No	Possible	Yes	No
Anti-TRIM38	No	Uncertain	Yes	No
ASCA	No	Possible	Yes	No
Anti-calponin-3	No	Possible	Yes	No
Anti-gAChR	No	Possible	Yes	Possible
Anti-AQP1	No	Uncertain	Yes	No
Anti-AQP4	No	Yes	No	Yes
Anti-AQP5	No	Possible	No	No
Anti-cofilin-1, antialpha-enolase, anti-RGI2	Possible	Possible	Yes	No
Anti P-selectin	No	Possible	Yes	No
Anti-carbamylated proteins	No	Possible	Yes	No
Anti-moesin	No	Uncertain	Uncertain	No

Subsequently, the same authors searched these autoantibodies in 13 patients with primary SS and found anti-SP-1 in 54%, anti-CA6 in 54% and anti-PSP in 18%; having all the patients at least one of them. They also evaluated 29 patients with less than two years with idiopathic xerostomia and xerophthalmia who met at least three diagnostic criteria for SS and all the exclusion criteria, and found that the prevalence of anti-SP-1 and anti-CA6 antibodies was 76%, while only 31% have anti-Ro/SSA and anti-La/SSB antibodies. They also observed that among 20 primary SS patients with positive salivary gland biopsies, but who lacked antibodies to Ro/SSA or La/SSB, anti-SP-1 and anti-CA6 antibodies were present in 45% and 5%, respectively [16].

On the other hand, in a cohort of 65 patients with idiopathic dry eye, the prevalence of anti-SP-1, anti-CA6 and anti-PSP antibodies was 60%, while only 30% of the patients were positive for anti-Ro/SSA and anti-La/SSB. Moreover, in this study, patients with disease duration of less than 2 years and mild xerophthalmia were all negative for anti-Ro/SSA and anti-La/SSB antibodies, but 25% had anti-SP-1 antibodies [17].

Suresh et al using sera from the Sjögren's International Collaborative Clinical Alliance Cohort (SICCA), a prospective cohort that included patients with sicca symptoms or with suspicion of SS, found that 67% of the patients with a focus score (FS) of zero were positive for anti-SP-1, anti-CA6 and anti-PSP antibodies while only 22% had anti-Ro/SSA and anti-La/SSB antibodies. In contrast, 80% of the patients with mild (FS $<$ 1 per 4 mm2) or moderate disease (FS $>$ 3 per 4 mm2) had either anti-Ro/SSA and anti-La/SSB antibodies, and 60% expressed either anti-SP-1, anti-CA6 or anti-PSP antibodies [18].

Furthermore, two large cohorts of primary SS patients have reported the prevalence of anti-SP-1 antibodies [19,20]. At the Greek cohort (n = 123) the prevalence was 52% and patients with a FS $\geq$ 2 were less likely to be anti-SP-1 positive [20], whereas in the Chinese one (n = 134), the prevalence was 40% and positive patients were more likely to have a FS = 0 and normal salivary gland function [21].

2.2. Anti-interferon-inducible protein-16 antibodies

Interferon-inducible protein-16 (IFI16) belongs to the HIN200/Ifi200 family of IFN- inducible genes, which is implicated in the pathogenesis of several autoimmune diseases through the overexpression of pro-inflammatory cytokines. In addition, IFI16 might be recognized as an autoantigen due to its mislocalization in the extracellular milieu [21,22]. Uchida and cols first reported the presence of autoantibodies against IFI16 in patients with SS. They found anti-IFI16 antibodies in 70% of 30 patients with primary SS, 13% of patients with rheumatoid arthritis (RA) and 33% of patients with systemic lupus erythematosus (SLE) [23]. Mondini and cols observed the same prevalence also in patients with primary SS [24]. Two subsequent studies described that anti-IFI16 antibodies were significantly more prevalent in patients with primary SS than in healthy controls (prevalence: 34% vs. 5% and 29% vs. 2.1% respectively) [23,24]. One of these studies did not find an association with any clinical or serological features [21], whereas in the other, anti- IFI16 antibodies were associated with abnormal Schirmer's test, hyperglobulinemia, ANA $\geq$1:320, presence of germinal center-like structures and a higher($>$ 3) FS in salivary gland biopsies, suggesting a more severe disease [22].

2.3. Anti-MDM2 antibodies

The human homologue of the mouse double minute 2 (MDM2) is considered an inhibitor of the tumor-suppressor protein p53 and the retinoblastoma protein. Liu and cols, reported an increased expression of MDM2 in an animal model of SLE and among patients with SLE [25]. The same group of investigators evaluated sera from 100 primary SS patients and 74 healthy controls and found significantly higher frequency of these autoantibodies in the former group (21% vs. 5.4%). Patients positive for anti-MDM2 antibodies were characterized by longer disease duration, more lymphocyte focal gathering in minor salivary glands, a higher prevalence of anemia, thrombocytopenia and anti-La/SSB positivity. Moreover, the titers of this antibody correlated with the ESSDAI and IgG levels. Interestingly, among 18 patients who were anti-Ro/SSA and anti-La/SSB negative, two of them had anti-MDM2 antibodies [26].

2.4. Anti-NA-14 antibodies

Nuclear autoantigen 14 KDa (NA-14) was identified as a novel autoantigen at the serum from a patient with SS [27]. Subsequently, Nozawa and cols, screened for anti-NA-14 antibodies among diverse autoimmune diseases. They found it in 18/132 (13.6%) patients with primary SS, 0/50 (0%) with secondary SS and in less than 4% with SLE, systemic sclerosis, RA, inflammatory myopathies and healthy controls. Furthermore, they observed that 36.3% of anti-NA-14 positive sera were negative for anti-Ro/SSA and anti-La/SSB [28]. Later, the same group of investigators in another study replicated a similar prevalence (11.1%), and described that anti-NA-14 positive patients have higher levels of IgA and tended to be ANA negative. In addition, the disease duration was shorter among anti-NA-14 positive patients, although it did not reach statistical significance [29].

2.5. Anti-stathmin-4 antibodies

Stathmins are a group of four phosphoproteins, which are part of the centrosome, the principal microtubule-organizing center of animal cells. Stathmin 1 is considered ubiquitous, whereas stathmin 2-4 are mostly expressed in the nervous system [30]. Duda and cols, attempted to search for potential autoantibodies in patients with SS complicated by polyneuropathy using high-density protein array, and found anti-stathmin-4 antibodies. Next, they developed an ELISA test which was carried out in 72 patients with primary SS and a diverse array of controls, such as secondary SS, SLE, RA, granulomatosis with polyangiitis, undifferentiated connective tissue disease, multiple sclerosis, metabolic and toxic polyneuropathy, multiple myeloma and healthy controls. They found anti-stathmin-4 antibodies of the IgG3 subclass in 15% of primary SS patients, and these antibodies were more frequent in patients with polyneuropathy (33% vs. 7.8%) and vasculitis (45% vs. 13%). Moreover, they were present as well in up to 18% of RA and 15% of secondary SS patients [31].

As stathmin-4 can be found within neurons and glial cells [30], and as stathmin deficient mice develop an age-dependent axonopathy of the central and peripheral nervous system [32], anti-stathmin-4 antibodies might play a direct role on nerve damage in patients with SS.

2.6. Anti-PUF60 antibodies

Recently, Fiorentino and cols identified a novel autoantibody by immunoblotting HeLa lysates of sera from patients with dermatomyositis (DM). The target of this autoantibody was characterized using a combination of human protein array profiling and mass spectrometry as PUF60 protein (poly(U)-binding splicing factor 60 kDa). Then, the same researchers determined the prevalence of anti-PUF60 antibodies in patients with DM and other autoimmune diseases, including SS, and healthy controls. They found anti-PUF60 antibodies in 25/84 (29.8%) SS patients, 48/267 (18%) DM patients, 6/71 (8.5%) SLE patients, 4/45 (8.9%) inclusion body myositis patients, 5/45 (11.1%) polymyositis patients and in 2/38 (5.3%) of healthy controls. Anti-PUF60 positive SS patients were more likely to be Asian or African descent and had a higher prevalence of ANA and RF, hypergammaglobulinemia and antithyroid antibodies; furthermore, anti-PUF60 antibodies were associated with the presence of anti-Ro52, anti-Ro60 and anti-La/SSB antibodies [33].

2.7. Anti-NR2 antibodies

NR2 receptors are expressed throughout the brain, mostly in the hippocampus, which has an important role in learning and memory function. Antibodies against NR1 are the cause of a well-characterized organ-specific autoimmune disease, namely, anti-NMDA receptor encephalitis [34]. Moreover, antibodies against NR2 have been widely studied in the context of neuropsychiatric SLE, with conflicting results [35]. Only one study has evaluated the presence of this antibody in primary SS. The authors tested for anti-NR2 antibodies in serum and cerebrospinal fluid (CSF) among 66 patients with primary SS and 66 healthy controls, and assessed the influence of these autoantibodies in memory function, depression and hippocampal volume. They found anti-NR2 antibodies in 13 (20%) SS patients in sera and in 6/51 (12%) patients in CSF. Patients with anti-NR2 antibodies in CSF had a worse performance in a set of eight of ten memory and learning tests, whereas those with serum anti-NR2 antibodies had a worse performance in six of the same tests. Additionally, more patients with depression were anti-NR2-positive (35% vs. 13%) [36]. In a subsequent study, the same authors also described that in a group of patients with primary SS (n = 50) and SLE (n = 50), those who were anti- NR2-positive in CSF, also had less hippocampal gray matter [37].

2.8. Anti-TRIM38 antibodies

Autoantibodies against Ro52 (tripartite motif [TRIM] containing protein 21 [TRIM21]) are present in almost 70% of patients with primary SS. Wolska et al detected autoantibodies reactive to another TRIM protein: TRIM38. Anti-TRIM38 antibodies were present in sera from 24/235 (10.21%) primary SS patients and 2/50 (4%) healthy controls. When they compared clinical and serological features between anti-TRIM38-positive patients vs. anti-TRIM38 negative patients, the former group had higher van Bijsterveld scores, lower Schirmer's test score, higher frequency of FS ≥ 3 and presence of anti-Ro52, anti-Ro60, anti-La, RF and hypergammaglobulinemia [38].

2.9. Anti-Saccharomyces cerevisiae antibodies

Anti-*Saccharomyces cerevisiae* antibodies (ASCA) are markers of inflammatory bowel disease, especially Crohn's disease where they are thought to be pathogenic. Nevertheless, ASCA might be present in other autoimmune diseases such as SLE, ankylosing spondylitis and RA [39]. Alunno and cols seeked to determine the prevalence and clinical associations of ASCA in a cohort of 104 patients with primary SS. They found a low prevalence (5%), and patients ASCA positive were more likely to have triple positivity for anti-Ro52/SSA, anti-Ro60/SSA and anti-La/SSB antibodies, hypocomplementemia and cutaneous and pulmonary involvement [40]. Interestingly, in their study, Alunno and cols also found high similarity between *S. cerevisiae* mannan and the 60kD Ro/SSA ribonucleoprotein, suggesting a possible pathogenic link via molecular mimicry [40].

2.10. Anti-calponin-3 antibodies

Birbaum and cols found a prominent 40 kDa band (calponin-3) when immunoblots were performed using serum from a SS patient and lysate from rat dorsal root ganglia. Next, they developed and ELISA assay to test for anti-calponin-3 antibodies in a cohort of patients with primary SS (n = 209), SLE (n = 138), myositis (n = 138), multiple sclerosis (n = 44) and healthy controls (n = 46). They found a prevalence of 11%, 8.7%, 5.1%, 6.8% and 2.2%, respectively. SS patients with peripheral neuropathy had more frequently these autoantibodies vs. healthy controls (17.9% vs. 2.2%), although this finding did not reach a statistically significant difference. Using immunohistochemistry, they also found that calponin-3 was widely expressed in perineural satellite cells in rat dorsal root ganglia, suggesting a potential pathogenic role of this novel autoantibody [41].

2.11. Anti-ganglionic acetylcholine receptor antibodies

The ganglionic nicotinic acetylcholine receptor mediates fast synaptic transmission in all peripheral autonomic ganglia. Anti-ganglionic acetylcholine receptor (gAChR) antibodies are found in half of the patients with autoimmune autonomic ganglionopathy (especially against the α3 and β4 subunits), a rare acquired immune-mediated channelopathy that leads to dysautonomia [42]. As autonomic dysfunction is a manifestation of SS, Mukaino and cols tested for the presence of anti-gAChRα3 and anti-gAChR β4 antibodies in patients with primary and secondary SS blinded to the presence or absence of autonomic dysfunction. They found positivity in 9 out of 39 (23%) patients, five of which had autonomic symptoms. Next, they tested both autoantibodies in ten patients with SS with known autonomic neuropathy and found positivity in eight of them (80%) [43].

2.12. Anti-aquaporin antibodies

Aquaporins (AQPs) are a family of 13 small proteins expressed at plasma membranes in many cells types that transport water and some small solutes like glycerol across cell membranes. They are widely expressed in the body, mainly in cells that are involved in fluid transport such as epithelial cells. Some AQPs have been detected in lacrimal and salivary glands [44]. Furthermore, studies have shown defective functions of AQP1 and AQP5 in patients with SS [45,46].

The most well-known AQP antibody is anti-AQP4 due to its pathogenic and diagnostic role in neuromyelitis optica spectrum disorder (NMOSD). The relationship of NMOSD and SS have been well described since the last decade [47].

Given that AQP4 are expressed in tissues affected in SS (salivary glands, lung, kidney) [44] and that anti-AQP4 antibodies are generated outside the central nervous system (CNS), anti-AQP4 antibodies could target those tissues. Javed et al performed minor salivary gland biopsies in 12 patients with classic Devic's disease (longitudinally extensive transverse myelitis [LETM] and optic neuritis) and eight patients with LETM. They found that 80% had a positive labial biopsy according to the Chisholm and Mason grading system for SS and half of them were anti-AQP4 positive. Two patients with LETM and two patients with Devic's disease satisfied criteria for Sjögren's disease [48]. These findings could imply that inflammation in minor salivary glands in the absence of overt SS in patients with NMOSD could reflect direct attack by anti-AQ4 antibodies. However, these autoantibodies have not been detected in sera of patients with SS without NMOSD features [47,49].

Birnbaum and cols determined the frequency of anti-AQP4 antibodies in a single- center cohort of 109 SS patients, including 11 with NMOSD, eight with non-NMOSD demyelinating syndromes and 90 without demyelinating syndromes. They found that only the 11 patients with NMOSD had positive anti-AQP4 antibodies (prevalence: 10%), a finding that implies syndrome-specificity for these autoantibodies and that the coexistence of NMOSD in SS is a true overlap syndrome and not a CNS manifestation attributable to SS [50].

On the other hand, AQP5 has an important role in saliva and tear secretion [44]. Transgenic mice lacking AQP5 produce hypertonic and viscous saliva [51] and AQP5 is diminished in lacrimal glands of SS patients [45]. Hence, Alam and cols hypothesized that SS patients may have autoantibodies against AQP5. They developed a cell-based indirect immunofluorescence (IIF) assay to detect these autoantibodies in a cohort of 112 primary SS patients and found a prevalence of 73.2% for the IgG and 13.4% IgA isotypes, with a sensitivity of 73% and specificity of 68%. The presence of anti-AQP5 IgG was associated with low resting salivary flow [52]. Following this study, the same authors described that anti-AQP5 antibodies from SS patients recognize distinct functional epitopes of AQP5, and thus, they may act as mediators of glandular hypofunction [54]. Moreover, the same group of

investigators using a cell-based IIF assay detected anti-AQP1 antibodies in 27.7% of 112 SS patients (20.5% for IgG and 7.1% for IgA); and contrary to anti-AQP5 antibodies, the presence of anti-AQP1 antibodies was not associated with low salivary flow [46].

Finally, Tzartos and cols searched for distinct antibodies against several AQPs in a cohort of 34 SS patients without neurological manifestations and found antibodies to extracellular domains of AQPs in 38.2% of them, two to AQP1, one to AQP3, six to AQP8 and four to AQP9, and none had AQP4 and AQP5 antibodies. When they compared AQP-positive versus AQP-negative patients, the former group had a slightly higher median Van Bijsterveld score [55].

2.13. Antibodies against P-selectin

P-selectin plays an important role in thrombosis and prothrombotic states. In a study, that include 32 patients with idiopathic thrombocytopenic purpura (ITP), 70 patients with primary SS with/without thrombocytopenia, and 32 healthy controls, the presence of plasma P-selectin autoantibodies were increased in primary SS patients with thrombocytopenia as compared to ITP patients, primary SS patients without thrombocytopenia, and healthy controls. Additionally, being positive for P- selectin autoantibodies was associated with a low platelet count [56].

2.14. Anti-carbamylated antibodies

Carbamylated proteins are present in several inflammatory process, suggesting that post-translational modifications may drive autoantibody production. Among a study of 84 patients with primary SS, 27% were positive for anti-CarP IgG antibodies. Levels of anti-CarP correlated positively with total IgG, IgM, RF, β2- microglobulins, as well as with the focus score and the presence of germinal center- like structures in the minor salivary glands [57].

2.15. Anti-moesin antibodies

Moesin is a structural protein involved in cytoskeletal organization and signaling pathway. Recently, it was identified as a novel autoantigen by Western blotting and immunoprecipitation in SS. Using ELISA methodology, 21 out of 50 Chinese patients with primary SS (42. %), 11 out of 50 patients with SLE (22%) and 2 out of 50 healthy controls (4%) showed anti-moesin antibodies positivity [58].

2.16. Anti-cofilin-1, anti-alpha-enolase and anti-RGI2 antibodies

Research oriented to the identification of predictors of malignant lymphoma is a challenge in SS [59]. In this sense, recently Cui and cols identified three novel autoantibodies using proteomic techniques with the purpose of finding biomarkers for primary SS and mucosa-associated lymphoid tissue (MALT) lymphoma. First, they identified target antigens in parotid gland tissue samples from patients with primary SS, primary SS/MALT lymphoma and non-SS controls. They found three proteins that were significantly overexpressed in the first two groups, namely, cofilin-1, alpha-enolase and Rho GDP-dissociation inhibitor 2 (RGI2). Next, they developed ELISAs for detection of antibodies against these proteins and tested saliva of 50 primary SS patients, 20 primary SS/MALT patients and 50 healthy control subjects. They observed that the level of the three autoantibodies were over-expressed in primary SS/MALT patients compared to primary SS patients and healthy controls, and that primary SS patients had higher levels compared to healthy controls as well. Receiver operating characteristic curve analysis revealed that the combination of the three autoantibodies were highly useful for the distinction of primary SS and primary SS/MALT patients vs. controls and for the distinction of primary SS/MALT vs. primary SS patients [60]. As cofilin-1, alpha- enolase and RGI2 might promote carcinogenesis and/or promote invasion and metastasis of cancer cells, the authors hypothesized that the overexpression of these proteins and their respective autoantibodies may play a role in the progression of primary SS to MALT lymphoma [60].

On the other hand, a citrullinated α-enolase peptide (CEP-1) was identified as a major antigenic target of anti-citrullinated protein antibodies (ACPA) in patients with primary SS. In this study, Nezos and cols reported increased anti-CEP-1 antibody serum titers in 60% ACPA + primary SS patients vs. 41.7% ACPA + RA patients, whereas no reactivity was detected in ACPA- primary SS patients. Furthermore, the subgroup of patients with anti-CEP-1 antibodies was characterized by high urinary pH levels. As alpha enolase has been shown to be present in tubular epithelial cells in the kidney, the authors speculated that anti-CEP-1 could contribute to renal tubular dysfunction in SS [61]. In the same sense, our group also characterized α-enolase as a dominant antigen in citrullinated lysates of HEp- 2 cells in primary SS in comparison with RA and healthy subjects. The presence of IgA and IgG anti-citrullinated α-enolase was not associated with any clinical manifestation with the exception of the IgA isotype with anti-Ro/SSA antibodies [62].

3. Conclusions

The detection of autoantibodies is useful for establishing diagnosis, classification and prognosis in autoimmune diseases. The recognition of novel autoantibodies in SS has increased in the last years, opening a window of opportunity to denote particular stages of SS (including preclinical), to establish clinical phenotypes, and to predict long-term complications such as lymphoma. Their study is also important to elucidate new aspects of disease pathophysiology, and in the future to permit a phenotype-specific patient approach. Nevertheless, future large-scale validation studies are still needed to corroborate their true meaning.

Authors' contributions

All authors have contributed to the writing of the manuscript.

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